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EXAMINER	
WALICKA, MALGORZATA A	
ART UNIT	**PAPER NUMBER**
1652	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/29/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/808,717	SAN ET AL.	
	Examiner	Art Unit	
	Malgorzata A. Walicka	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 27- 33 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 27-33 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Response to Office Action filed Oct. 31, 2006 is acknowledged. Claims 1-26 have been cancelled; new claims 27-33 have been filed. The new claims read on the elected invention VIII, directed to a method of manipulation of metabolism of a cell comprising elevated expression of a combination of three enzymes, pantothenate kinase, alcohol acetyl transferase and pyruvate dehydrogenase. Claims 27-33 are under examination.

Detailed Action

Objections

Fig. 6 and Table 2 are objected to for confusing description of the E. coli transformed for producing ester isoamyl acetate. To produce isoamyl acetate E. coli has to be transformed with an acetyltransferase gene. In description of Fig. 6 the transformant used for production of acetate is named as DH10(pKmAT, pRV380), which according to Table 2 overexpresses only *panK*.

Rejections

Since claims 1-26 has been cancelled, all rejections made in the Office action of June 30, 2006 (previous action) are moot, unless applicable to the new claims.

U.S.C. 112, second paragraph

Claims 27-31 and 32-33 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

Claim 27 recites the limitation "increasing CoA production" in part b) of the claim. There is insufficient antecedent basis for this limitation in the claim. The preamble recites "manipulating of metabolism".

Claim 27 is not clear in reciting "manipulating the metabolism of a cell', which has a very large scope. Without indicating what the purpose of manipulation is the claim is indefinite. For examination purposes the examiner assumes the claim is directed to a method of "increasing CoA production".

Claim 27 is confusing. It is not clear to one having skills in the art, why the Applicants, aiming at the increase in CoA production in a transformed cell, transfect the cell with a pyruvate dehydrogenase gene and with ATF2 gene. According to the state of art at the time of filing, both enzymes deplete CoA pool. Pyruvate dehydrogenase uses CoA for production of acetyl-CoA and ATF2 uses acetyl-CoA for production of isoamyl acetate. See the documents in the examiner references, form 892. From genes (i), (ii) and (iii) recited by claim 27 only gene (i) encoding pantothenate kinase directly involved in production of CoA seems to serve the purpose of increasing CoA production. Applicants own data presented in Fig 7. indicate that transformation with a PDH gene does not increase CoA production.

Claims 32 lacks antecedent basis for the phrase "increasing CoA production", because the preamble recites "increasing coenzyme A (CoA) dependent metabolism".

Claim 32 is not clear in recitation "increasing Coenzyme A(CoA) dependent metabolism". Neither the claim not the specification defines the phrase "increasing CoA metabolism". CoA is involved in more than 100 biochemical reactions, thus referring to "increasing CoA metabolism" without indicating which reaction or reactions Applicants have in mind renders the claim indefinite. For examination purposes the examiner assumes the claim is directed to a method of "increasing CoA production" and increasing isoamyl acetate production.

Claim 32 is confusing as containing the term "isoamyl alcohol" at the end. For examination purposes it is assumed that Applicants mean "isoamyl acetate".

Claims 31 and 32 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential element, such omission amounting to a gap between the elements. See MPEP § 2172.01. The omitted element is addition of isoamyl alcohol to the growth medium, without which the isoamyl acetate cannot be produced, at least not in the transformants disclosed by Applicants.

Claims 29 and 33 are rejected for lack of antecedent for *atf2* gene. The *atf2* gene is not recited by the base claims from which claim 29 and 33 depend.

35 USC section 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which

it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Written description

Claim 27-33 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to the increasing CoA production in any bacterium transformed with a pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase gene wherein in addition ackA or pta or both ackA and pta genes have reduced activity and additionally wherein production of isoamyl acetate is increased in said transformants.

The specification teaches only E. coli cells transformed with panK, ATF2 and pyruvate dehydrogenase genes separately, or in combination; see Table 2. None of E. coli transformants disclosed by Applicants as possessing the there genes recited in the claim, i.e. transformants DH10B (pGS367, pATCA) and YBS121 (pATCA, pGS367) in Table 2, are shown to possess the feature of increased production of CoA in comparison with control; this is a new matter. The data presented in Fig. 5, 6a, and 6b concerns E. coli cells transformed with its own panK gene (according to description of plasmids and E. coli transformants in Table 2). Parts b)-d) of Fig. 6 seem to refer to

double mutants containing panK and ATF2. Fig. 7 presents the data for E. coli transformed with panK or panK and PDH gene.

Furthermore, certainly not any bacterium may be successfully transformed to attain the goal of an increase in production of CoA, and isoamyl acetate as claimed in claims 31 and 32-33, simply because bacteria are very versatile in their metabolism. It is doubtful that, for example, bacteria growing under extreme condition of low pH, high temperature and large concentrations of ferrum ions are suitable to make the transformants of the claimed method.

The origin of genes used for manipulation are not described by the claims. Also, the disclosure does not provide sufficient written description of transformation of any bacterial cell. For their transformation of E. coli applicants used E. coli panK gene and ATF2 gene form yeast, and a not identified pyruvate dehydrogenase gene obtained form yeast. Manipulating metabolism in E. coli cells by transforming them with panK, ATF2 and pyruvate dehydrogenase genes separately or in combination does not provide sufficient written description for transforming any cells with any generic gene or their combination as recited by the claims. The host cells have their specific requirements as to the expression vector, i.e., plasmid and control expression elements it comprises. Some genes are extremely difficult to be expressed in host different than the cell of origin, and require extensive modifications regarding, for example, their N-termini sequences as well as codons used. Moreover, those skilled in the art realize that metabolic pathways of every cell type are distinct, such that a showing that

overexpression of a group of genes in one cell type leads to increased level of CoA may not be true in other cell type.

Claims 28, 32 and 33 are rejected for lack of written description of a bacterium cell having reduced activity of *ackA* or *pta*. Applicants teach they used E. coli YBS121 double mutant *ackA-pta* mutant that has both genes inactivated. Neither the specification or the claims as originally filed teach a bacterium cell having reduced activity of *ackA* gene or *pta* gene separetely. This rejection is for new matter.

Furthermore, claims 31-33 are rejected for lack of written description of any bacterial cell that in result of transformation started to produce more isoamylacete, because the only bacterial cell the Applicants used for transformation, i.e. E. coli, does not produce isoamyl acetate itself. Thus, the production may not be increased in result of transformation, it may only start after transformation. This rejection is for new matter.

Applicants' attention is also turned to the fact that claim 31 suffers from lack of description of increasing production of isoamyl actetate by increasing CoA production. Applicants have not disclose a correlation between increasing CoA production and increasing production of isoamyl acetate. What is necessary for production of isoamyl acetate by E. coli is to transform E. coli with acetyl transferase gene and to grow the transformant in the presence of isoamyl alcohol. This rejection is for new matter.

All together, Applicants have failed to sufficiently describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize

Applicants were in possession of the claimed invention at the time the application was filed. What Applicant have described is:

1) E. coli transformed with gene (i) is producing more CoA then not transformed, when cultured in the presence of pantothenic acid; see Fig. 5 and 6a,

2) E. coli transformed with (i) and (iii) is able to produce isoamyl acetate, Fig. 6 and 10,

3) E. coli transformed with (i), (ii) and (iii) has higher production of isoamylacetate than the one transformed with (i) and (iii).

Applicants also have shown that addition to the culture of transformants of substrate for production of CoA i.e. pantothenic acid, and the substrate for production of isoamyl acetate, i.e., isoamyl alcohol, increases production of isoamyl acetate in transformant listed under 2) and 3) above; see Fig. 10.

3.2.2. Scope of enablement

Claim 27-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the claimed methods wherein E. coli is used as host cell to be transformed with the genes Applicants used, does not reasonably provide enablement for any bacterial host cell to be used in the claimed methods; see also the above rejection for lack of written description. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The nature and breadth of the claims covers using any bacterial cell for generating a transformant comprising pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase genes, wherein said transformant produces more CoA than its nontransformed counterpart, wherein additionally the host cell exhibits reduced activity of ackA, pta or ackA and pta genes, and wherein isoamyl acetate is produced.

While manipulating metabolism of bacterial cell by expressing recombinant enzymes is well developed and the skills of artisans high, not every gene encoding the enzymes listed generically by the claims may be expressed in any cells. Genetic code usage is specific for the cell to be transformed. The host cells have also specific requirements as to the expression vector, i.e., plasmid and control expression elements it comprises. Some genes for one organism require modification of N-terminus to be exprsssed in some bacteria. Furthermore, providing for E. coli transformed with panK or with panK plus dehydrogenase gene is not a sufficient guidance for the genus of genes and cells to be used by a skilled artisan to make the invention as claimed. In addition, Applicants' own data suggest that without supplying the growth medium with the pantothenic acid and/or isoamyl alcohol overexpressing panK and ATF alone is not sufficient for increase production of CoA and isoalmyl acetate. Moreover, those skilled in the art realize that metabolic pathways of cells of every cell type are distinct, such that a showing of overexpression of a group of genes in one cell type leads to increased level of CoA may not be true in other cell types as well.

Furthermore, Applicants do not enable a cell having reduced activity of ackA, pta or ackA and pta genes, because Applicants just used one E. coli natural mutant (YBS121) having activity of the ACKA and PTA enzymes reduced. The source of the mutant is not stated. Applicants do not teach the structure of E. coli ackA and pta genes and mutation(s) that reduce the activity of the encoded enzymes. Thus, reduction of ACKA and PTA activity is not enabled, imposing on the skilled artisan an experimentation that is undue.

Moreover, those skilled in the art realize that metabolic pathways of every cell type are distinct such that a showing overexpression of a group of genes in one cell type leads to increased the level of CoA may not be true in other cell types as well.

In addition, both the genus of genes and the genus of cells to be transformed, encompass an extremely large number of species and there is no teaching by Applicants which gene, expression vector and cell to chose to get a successful combination.

In summary, a skilled artisan, who would like to make and use the claimed invention is forced to experimentation that has a low probability of success without additional teaching as to bacterial cells and identifying genes that are to be used in the invention. In conclusion, to make and use the broadly claimed invention requires experimentation that is improperly extensive and undue.

35 USC section 102 and 103

Previously presented claims 1 and 3 were rejected in the Office Action of June 30, 2006 (previous action) under 35 USC section 102(b) as anticipated by San et al., (Metabolic Engineering through Cofactor Manipulation and Its Effects on Metabolic Flux Redistribution in *Escherichia coli*, Metabolic Engineering, February 27, 2002, 4, 182-192, included in the Information Disclosure Statement) and Rock et al. (Pantothenate Kinase Regulation of the Intracellular Concentration of Coenzyme A, J. Biol. Chem. 2000, 275, 1377-1383). Claim 4 was rejected as obvious over San et al. in the view of Rock. Because claims 1-4 are cancelled the rejections are moot. However, the examiner addresses Applicants traverse of the rejection of claims 1-4 over prior art.

On page 5 of 6 of their REMARKS Applicants present the position that rejection of claims 1 and 3 under 102(b), and for that reasons under 103, was not correct because:

1) the electronic version of San et al. was available only on May 16, 2002, which is after filing the priority document in this application, and

2) the article by San et al. is the work of the present inventor.

Regarding point 1) Applicants have not provided any proof that the electronic version of the article by San et al. was available only May 16, 2002 and not on February 27, 2002 as stated on the first page of the printed version of the article.

Regarding point 2), the instant inventors are San, Bennett and Vadali, whereas the authors on the disputed article are San, Bennett, Berrios-Rivera, Vadali, Yang, Horton, Rudolph, Sariyar and Blackwood. Thus, the article is not by inventors.

3.4. 35 USC 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 32-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over San et al. (Metabolic Engineering through Cofactor Manipulation and Its Effects on Metabolic Flux Redistribution in *Escherichia coli*, Metabolic Engineering, February 27, 2002, 4, 182-192, included in the Information Disclosure Statement), in view Vallari D. et al. (Biosynthesis and Degradation Both Contribute to the Regulation of Coenzyme A Content in *E. coli*, J. Bact, 1988, 3961-3966, sent to Applicants with the previous action) and Voet et al. (Biochemistry, second Edition, 1995, John Wiley & Sons, Inc, pp.543-548 enclosed), and Yang et al. (Effect of Inactivation of *nuo* and *ackA-pta* on Redistribution of Metabolic Fluxes in *Escherichia coli*, Biotech. Bioeng. 1999, 65, 291-297, enclosed).

The claims are directed to a method of increasing isoamyl acetate production in a bacterial cell transformed with a

(i) pantothenate kinase gene,

(ii) pyruvate dehydrogenase gene, and

(iii) alcohol acetyl transferase gene,

wherein the cell has reduced activity of *ackA*, or *pta* or both *ackA-pta*.

San et al. teach transformation of E. coli (a bacterial cell) having overexpressed panK or wild type expression of panK with ATF2 gene of S. cerevisiae; see strains DV79 and DV62 description on page 190, left column under subtitle *Result and Discussion*, and Table 3. Transformation of these lines with ATF2 gene alone causes production of isoamylacetate, in E. coli which by itself does not produce isoamyl acetate. Furthermore San et al. teach that overexpressing panK (the feature of strain DV79), causes greater production of isoamylacetate than in DV62 that does not overexpress panK. This fact was related by San et al. to an increase in the pull of CoA in DV79; see the text under Table 4 and Fig. 6(b). In addition, in Fig. 6(b) San et al. teach that acety-CoA is also used for production of acetate. However, San et al. do not teach

1) transformation of E. coli with panK gene,

2) transformation of E.coli with pyruvate dehydrogenase gene and

3) E. coli cell having *ackA-pta* reduced.

Vallari et al. 1988 teach that **the total CoA content in E. coli cells** depends on the **pantothenate kinase**; see the end of the abstract, and that the regulation of pantothenate kinase appears to be the most important determinant of the CoA synthesis rate; see introduction line 9. Vallari et al. teach also that panthotenate kinase is feedback inhibited by CoA.

Biochemistry handbook teaches on page 543 that acetyl–CcoA is synthesized from pyruvate and CoA by **pyruvate dehydrogenase**, page 543, Fig. 19.6, and the text under the figure. The handbook also teaches on page 547 teaches that the acetyl-AoA inhibits pyruvate dehydrogenase.

Yang et al. tech that two enzymes phosphotransacytylase and acetate kinase produce acetate from acetyl CoA, and E. coli YBS1219 (used in the instant invention) which has **inactivated *ackA-pta*** does not produce acetate (Table 4. page 295). This means that YBS1219 cells have a large pool of acetyl-CoA for production of chemicals other than acetate.

It would have been obvious for one having ordinary skills in the art who would like to produce isoamyl acetate in E. coli to transform E. coli (bacterial) cell with atf2 as Sun did, and replace an E. coli having overexpressed panK gene by a E. coli into which the pan K gene was introduced for elevating expression of the pantothenate kinase. The motivation is provided by Vallari et al. who teach that panK is the rate-controlling enzyme in CoA biosynthesis in E. coli. Thus, one having intention to produce isoamyl acetate in E. coli would have increased production of A-CoA as a precursor for isoamyl acetate production. The expectation of success was very high because the art at the time of filing taught it was easy to transform E. coli with its own gene as the Applicants did. Thus it was obvious to make E. coli transformed with atf2 and panK.

Further, it would also have been obvious for a skilled artisan who would like to produce isoamyl acetate in E. coli to transform E. coli with a gene encoding pyruvate

dehydrogenase, because, as expected from the handbook of biochemistry that would increase production of acetyl-CoA which is the substrate for isoamyl acetate.

Finally, because acetyl CoA is the substrate for isoamyl acetate the skilled artisan who would like to produce efficiently isoamyl acetate whould have been motivated to work with a cell having ackA-pta genes inactivated, because as Yang et al have shown this increases the pool of acetyl CoA available for production of isoamyl acetate.

In conclusion, the claimed invention was within the ordinary skill in the art to make and use at the time it was made and was as a whole, *prima facie* obvious.

Conclusion

All claims are rejected.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of

the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka whose telephone number is (571) 272-0944. The examiner can normally be reached on Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached on (571) 272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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